REMARKS

Status of the claims

With entry of the amendment, claims 29-33, 35-41, 43-48, and 55, 56, and 58-64 are pending in the application. In the non-final Office Action mailed July 14, 2004, claims 29-33, 35-38, 40, 41, 43-46, 48, and 55-57 were variously rejected and claims 39 and 47 were objected to as summarized below. In view of the amendments above and the arguments below, Applicants respectfully request reconsideration on the merits of the application, withdrawal of the rejections and objections, and allowance of the claims.

Summary of amendments

The specification was amended to delete the incorporation by reference at page 39, line 22. The amendment introduces no new matter.

Claims 1-28, 34, 36, 42, 49-54, and 57 are cancelled without prejudice to filing a continuation or divisional application thereon. Claims 1-28, 34, 42, and 49-54 were cancelled in a previous response. The listing of claims properly reflects the status of those claims. Claim 36, which indirectly depends from claim 29, has been cancelled in favor of new independent claim 62, which includes the limitations of claims 36 and 29. Claim 57 has been cancelled.

Claim 29 was amended to render its punctuation consistent with claim drafting conventions, specifically, to eliminate periods contained within the body of the claim. Claim 29 was amended to modify the transitional phrase to encompass methods using a first oligonucleotide "consisting essentially of" the recited elements. In addition, claim 29 was amended to clarify that the Template Hybridization Domain consists of a sequence of about 5 to about 20 nucleotides (original claim 29) having 5 or more bases complementary to the Substrate Hybridization Domain bases (page 22, lines 25-27). Step (b) of claim 29 was amended to clarify that the method produces an oligonucleotide probe (page 11, lines 19-22). Further, claim 29 was amended to clarify that the Target Binding Domain is heterologous to the Signal Binding Domain of the first oligonucleotide (Template Nucleic Acid) (page 10, lines 17-21).

Claim 32 was amended to modify the transitional phrase to encompass methods using a second oligonucleotide "consisting essentially of" the recited elements.

Claim 40 was amended to modify the transitional phrase to encompass methods using a Signal Template Domain "consisting essentially of" the recited elements.

Claim 41, which recited the degree to which the Signal Domain is homopolymeric as a number of alternative percentages (at least 50%, at least 70%, at least 90%, or 100%) has been amended to recite a Signal Domain that is at least 50% homopolymeric. New claims 58-60 have been added to recite a Signal Domain that is at least 70%, at least 90%, or 100% homopolymeric, respectively.

Claim 43, which depended from claim 29, has been amended to depend from claim 41.

Claims 55 and 56, which depend from claim 29, were amended to delete the recitation of the elements of the Probe because such recitation is redundant in view of the recitation of those same elements in claim 29.

New claim 61 includes a limitation to a first oligonucleotide, equivalent to a Template Nucleic Acid (page 10, lines 8-11), consisting essentially of a sequence of about 10 to about 120 nucleotides (page 5, lines 16-19).

New claim 62 includes a limitation to a second oligonucleotide, equivalent to a Substrate Nucleic Acid (page 10, lines 8-11), consisting essentially of a sequence of about 15 to about 150 nucleotides (page 18, lines 26-27).

New independent claim 64 contains all of the limitations of claim 29, but requires that the Substrate Hybridization Domain consist of a sequence of from about 5 to about 10 nucleotides and that the Template Hybridization Domain consist of a sequence of from about 5 to about 10 nucleotides (page 19, line 6).

The amendments are fully supported by the application as filed and introduce no new matter.

Objection to the specification

The specification has been objected to for the citation of documents that are allegedly improperly incorporated by reference. Applicants have deleted the incorporation by reference at page 39, line 22 and respectfully request that the objection be withdrawn. Applicants concur with the Examiner's tacit acknowledgement that the incorporation of those references cited in the application is not necessary to satisfy the written description or enablement requirements in that the claims are fully described and enabled by the specification without reference to any of the cited references.

Rejection under 35 U.S.C. 112, second paragraph

Claims 55 and 56, which depend from claim 29, are rejected under 35 U.S.C. 112, second paragraph as being indefinite for the recitation of "Probe", for which there is no

antecedent basis in claim 29. Claim 29 has been amended to clarify that the oligonucleotide synthesized by the method of the invention is an oligonucleotide Probe. In view of the clarifying amendment, Applicants respectfully request that the rejection be withdrawn.

Rejections under 35 U.S.C. 103(a)

Independent claim 29 and claims 29-33, 35-38, 40, 41, 43-46, 48, 55, and 56, which depend directly or indirectly from claim 29, were rejected under 35 U.S.C. 103(a) as being unpatentable over US Patent No. 5,882,856 (Shuber et al.) in view of US Patent No. 5,710,028 (Eyal et al.). Claims 39 and 47 are deemed to be free of the prior art and patentable, but are objected to as depending from a rejected base claim (claims 29 and 46, respectively).

As a preliminary matter, Applicants have amended claim 29 to recapture the claim scope to which Applicants are entitled, specifically, to reflect that the Substrate and Template Hybridization Domains are from about 5 to about 20 nucleotides. In Amendments dated October 29, 2002 (Paper 18) and March 1, 2004 (Paper 20), Applicants amended claim 29 to further limit the size of the Substrate and Template Hybridization Domains. However, Applicants respectfully submit that, as explained in detail below, a limitation as to the size of the Hybridization Domains is not necessary to distinguish the claims over the prior art.

Independent claim 29, as currently amended, is reproduced below:

29. (Currently Amended) A method of labeling an oligonucleotide, comprising the steps of:

(a)[.] hybridizing a first oligonucleotide to a second oligonucleotide, wherein the first oligonucleotide consists essentially of ~~comprises~~, from 3' to 5': a Substrate Hybridization Domain adjoining a Signal Template Domain, wherein:

i)[.] the Substrate Hybridization Domain consists essentially of a sequence of about 5 to about ~~10~~ 20 nucleotides; and

ii)[.] the Signal Template Domain consists of a sequence of about 5 to about 100 nucleotides;

and the second oligonucleotide comprises, from 3' to 5': a Template Hybridization Domain adjoining a Target Binding Domain, wherein:

i)[.] the Template Hybridization Domain consists essentially

of a sequence of about 5 to about ~~10~~ 20 nucleotides[,] which is not detectably labeled, and which has 5 or more bases complementary ~~shows~~ ~~complementarity~~ toward and is hybridizable to the Substrate Hybridization Domain of the first oligonucleotide; and

ii)[.] the Target Binding Domain is not detectably labeled and comprises a nucleotide sequence heterologous to that of the Template Hybridization Domain and to that of the first oligonucleotide; and[:]

(b)[.] extending the second oligonucleotide with a DNA polymerase in the presence of a labeled nucleotides to create an oligonucleotide Probe having from 5' to 3' an unlabeled Target Binding Domain adjoining a Template Hybridization Domain adjoining a labeled Signal Domain.

Applicants respectfully submit that the Examiner has not established a *prima facie* case of obviousness, which requires: (1) some suggestion or motivation, either in the references themselves or in the knowledge generally available to one of ordinary skill in the art, to modify the reference or to combine reference teachings; (2) a reasonable expectation of success; and (3) the art reference or combination of references must teach all of the claim limitations (MPEP 2142). The teaching or suggestion to make the claimed combination and the reasonable expectation of success must both be found in the prior art, not in applicant's disclosure. *In re Vaeck*, 947 F.2d 488, 20 USPQ2d 1438 (Fed. Cir. 1991) (MPEP 2143).

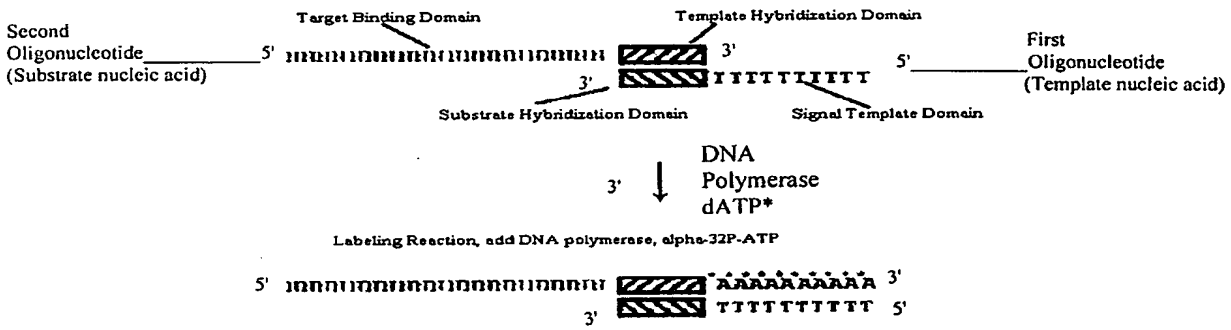
As explained below, the cited references, whether alone or in combination, fail to teach all of the claim limitations.

In one aspect, the invention includes methods of labeling the 3' end of an oligonucleotide to produce a detectably labeled oligonucleotide probe having a high specific activity.

As diagrammed below, the method involves hybridizing two oligonucleotides together through short hybridization domains of about 5 to about 20 nucleotides. The first oligonucleotide or "Template Nucleic Acid", which consists essentially of a Substrate Hybridization Domain of about 5 to about 20 nucleotides at or near its 3' end adjoining a 5' Signal Template Domain of about 5 to about 100 nucleotides, is hybridized to the second oligonucleotide (referred to as "Substrate Nucleic Acid" in the application), which comprises

a Template Hybridization Domain consisting essentially of about 5 to about 20 nucleotides at or near its 3' end, adjoining a heterologous 5' Target Binding Domain, through base pairing between five or more nucleotides of their respective hybridization domains. The first oligonucleotide or Template Nucleic Acid has a sequence that is independent of and heterologous to that of the Target Binding Domain, and thus serves as a universal nucleic acid in that it can be used to label any Substrate Nucleic Acid of interest (page 10, lines 17-21). Following hybridization, a DNA polymerase catalyzes the addition of detectably labeled nucleotides to the 3' end of the second oligonucleotide or Substrate Nucleic Acid to form a detectably labeled oligonucleotide probe that can be used to detect sequences complementary to the 5' Target Binding Domain .

The labeled oligonucleotide Probe thus produced comprises, from 5' to 3', a Target Binding Domain, a Template Hybridization Domain, and a labeled Signal Domain. The Probe can be used in a variety of assays, including, but not limited to, electrophoretic mobility shift assays, Northern or Southern analysis, and the like (page 9, line 34-page 10, line 2).



In contrast to the claimed invention, Shuber discloses a method of simultaneous PCR amplification of multiple target DNA sequences, each of which requires a DNA primer pair. According to Shuber et al., the 3' half of each primer comprises a target-specific sequence present or potentially present in the target DNA or its complement (column 4, lines 49-51).

The Examiner asserted that Shuber discloses the use of a chimeric primer configured as 5' -XY-3', with the "X" domain comprising a sequence that does not hybridize to the target sequence and the "Y" domain comprising a sequence contained within or flanking the target sequence or its complement. The Examiner therefore concluded that the "X" domain of Shuber's primer meets the limitations of applicants' Signal Template Domain and the "Y" domain meets the limitations of applicants' Substrate Hybridization Domain of the first

oligonucleotide. The Examiner further concluded that Shuber's "target sequence" meets the limitations of applicant's second nucleic acid sequence.

Contrary to the Examiner's assertion that the first oligonucleotide of the presently claimed invention has the same configuration as that used in the methods of Shuber et al., the 3' end of the first oligonucleotide (Template Nucleic Acid) of claim 29 does not comprise a sequence contained within or flanking the target sequence or its complement. The Examiner appears to suggest that because the 3' Substrate Hybridization Domain is complementary to the Template Binding Domain, and the Template Binding Domain flanks the Target Binding Domain, Shuber et al. teaches the first oligonucleotide. However, claim 29 and its dependent claims require that the Substrate Hybridization Domain of the first oligonucleotide is heterologous to the target sequence that "the Substrate Hybridization Domain does not follow the Target Binding Domain in its native context" (page 10, lines 17-21). In contrast, Shuber et al. does not teach or suggest that a sequence flanking the target sequence is heterologous in that it does not follow the target sequence in its native context.

The primary objective of Shuber et al. was to develop methods that would allow the amplification of specific gene sequences of interest from genomic DNA in a multiplex reaction. Therefore, the primer of Shuber et al. hybridizes to the genomic target sequence through a 3' sequence complementary to the target or flanking sequence and facilitates amplification of the target sequence by 3' extension of the primer. In contrast, the first oligonucelotide of claim 29 is a universal template in that its interaction with the second nucleic acid occurs through complementary hybridization domains with sequences heterologous to and independent of the target sequence.

The second oligonucleotide (Substrate Nucleic Acid) has a 5'-YX-3' configuration, with the 5' Y domain comprising a target sequence and the 3' X domain comprising a Template Hybridization Domain capable of hybridizing to the 3' Substrate Hybridization Domain of the first oligonucleotide. Extension of the second oligonucleotide in the presence of labeled nucleotides results in the addition of bases complementary to the 5' Signal Domain of the first oligonucleotide to form a labeled oligonucleotide probe.

Applicants submit that neither the first or second oligonucleotide of claim 29 would be capable of performing the method of Shuber et al. If one attempted to use the first oligonucleotide of claim 29, which is heterologous to the target sequence, no hybridization, and therefore, no amplification, would occur. As for the second oligonucleotide, the 5' end of the primer would hybridize to the target, but the 3' end would not. Therefore, a DNA

polymerase would be unable to catalyze the amplification of the target sequence to produce PCR products.

Similarly, substituation of either the first or second oligonucleotide of claim 29 for the primer or target disclosed in Shuber et al. would not result in the formation of a labeled oligonucleotide having, from 5' to 3', a Target Domain adjoined to a Template Hybridization Domain heterologous to the target, adjoined to a labeled Signal Domain, as required by step (b) of claim 29.

As the Examiner acknowledged, Shuber et al. does not teach or suggest using RNA nor incorporation of labeled nucleotides. Applicants respectfully submit that Shuber et al. did not disclose incorporation of labeled nucleotides precisely because the method was intended to produce multiple copies of target sequences through PCR amplification for use in subsequent analysis, not to make a labeled oligonucleotide probe according to the present invention.

The Examiner, citing column 3 of Eyal et al., characterized Eyal et al. as teaching "incorporation of detectably-labeled nucleotides at the terminus of a primer to label a nucleic acid molecule as well as to detect a target nucleic acid in a sample."

Applicants respectfully assert that Eyal et al. does not cure the deficiencies of the Shuber et al. in that Shuber et al. and Eyal et al. do not combine to teach or suggest all of the limitations of the claimed invention. The discussion at column 3 relates to detecting a point (single base) mutation in genomic DNA by PCR amplification using primers that are complementary to the sequence immediately adjacent to the site of the base under evaluation using radioactively or fluorescently labeled primers or nucleotides.

Eyal et al. provides a method of detecting point mutations using target specific primers designed to hybridize immediately adjacent to the base at which a point mutation may occur. The method employs labeled dideoxynucleotides, the incorporation of which into a primer extension product results in termination of the extension after the addition of only a single base. The extended primer of Eyal et al. is not a Probe within the meaning of the presently claimed invention, which defines a Probe as the labeled nucleic acid generated by extension of the Substrate Nucleic Acid (page 11, lines 19-21). The first oligonucleotide of claim 29, which serves as a template in making the Probe, is heterologous to the Target Binding Domain of the second oligonucleotide of claim 29, and therefore directs incorporation of labeled bases to form a labeled oligonucleotide having a Signal Domain that

is heterologous to the target. Eyal et al. does not teach or suggest using their primer, which is extended by a single labeled dideoxynucleotide, as a Probe. Rather, Eyal et al. provides methods for detecting the presence or absence of a point mutation by identifying the dideoxynucleotide incorporated into a primer opposite a specific base on the template target DNA. Further the method taught by Eyal et al. results in incorporation of a single label, whereas the method of the present invention results in the the incorporation of multiple label groups to achieve signal amplification.

The Office Action noted that Eyal et al. stated that their method is "simple, rapid, and highly accurate." However, the statement does not provide motivation to modify Shuber et al. Shuber et al. is directed toward methods of amplifying multiple target sequence in a multiplex PCR reaction to obtain products for subsequent analysis. In contrast, the methods of Eyal et al. involve the capture of an extension product terminated at a predetermined base after the addition of a single labeled dideoxynucleotide to permit identification of the base at that specific position. In any event, even if one were motivated to combine the references, the combination of references fail to teach every claim limitation.

Accordingly, Applicants respectfully request that the rejections under 35 U.S.C. 103(a) be withdrawn.

As the application is now in condition for allowance, Applicant requests allowance of the claims. Should the Examiner feel that any other point requires consideration or that the form of the claims can be improved, the Examiner is invited to contact the undersigned at the number listed below.

Applicant notes that this response is being submitted within three months of the expiration of the shortened statutory period for filing a timely response. This response is accompanied by a Petition for a three-month extension of time and check No. 52344 in the amount of $510.00 to cover the fee associated with the petition.

Please charge any additional fee due or credit any overpayment of fees to Deposit Account No. 50-0842.

Respectfully submitted,

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